# Inoculum Sources and Preservation in Soils of *Phytophthora parasitica* from Cherry Tomato in Continental Crop Areas in Southeast Spain

M. de Cara, M. Pérez-Vargas, M. Santos-Hernández and J.C. Tello-Marquina
Departamento Producción Vegetal
Universidad de Almería
Ctra. Sacramento s/n.
04120 Almería
Spain

D. Palmero
Escuela Universitaria de Ingeniería Técnica Agrícola
Ciudad Universitaria s/n.
28040 Madrid
Spain

J. Gómez-Vázquez
Instituto de Investigación y Formación Agraria y Pesquera
Camino San Nicolás s/n.
La Mojonera
Spain

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**Abstract**

**Cherry tomato crops were introduced in the late 1990s in the continental areas of southeast Spain. These fields had been previously cultivated with dry land crops as grapevine, olive, and cereal. After two years of cultivation, different soil-borne diseases widely appeared. The main disease observed was the root rot caused by *Phytophthora parasitica*, killing the plants during harvest period, concurring with the maximum demand of water from plants. The importance of the mycosis in the area together with the lack of control were the aim to first search for the inoculum sources, and then study the preservation of this oomycete in the infested soils for a long period time. Regarding the inoculum sources, no *Phytophthora* was found in seeds or seedlings from commercial nurseries sampled from the studied area, but the pathogen was isolated from the irrigation pools. *Phytophthora parasitica* was also isolated from the soils of the home gardens within the surrounded area, and even from the wheels of the tractors used in these fields. About the preservation study, a total of 92 samples from 42 different fields naturally infested with *P. parasitica* were analysed. All samples have been kept under laboratory conditions in sealed plastic bags. Only 20.58% of all samples preserved the oomycete for 4 years (48 months), and 18.18% for 5 years (57 months). These results can explain the rapid dissemination of the disease and its difficult control in the area.**

## INTRODUCTION

The surface devoted to cherry tomato in the inner areas of the province of Granada (Spain) is 170 ha up and it is currently increasing due to the high profitability of the crop, that allows growers to close the whole year European market with a local summer production (Berenguer et al., 2001). These summer crops started in 1999, obtaining high yields until the second year repeating the crop. After the second year plant losses were observed and many fields showed plants affected by wilt and rot root that in some cases reaches to the total of the field. Several surveys were carried out, and the most prevalent pathogen found was *Phytophthora parasitica*. This ooomycete is a soil-borne pathogen that is more infective with high soil moisture, thanks to the production of zoospores; and can resist adverse environmental conditions thanks to the production of chlamydospores (Fig. 1) (Erwin and Ribeiro, 1996). These features together with the importance of the disease in the area (65% of the surveyed fields), makes necessary an epidemiological knowledge of the pathogen in order to establish a successful control of the disease. With this purpose, the primary inoculum sources for the oomycete and its potential of preservation in soil has been studied in the present work.

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## MATERIALS AND METHODS

### Inoculum Sources

Because the fields were in new areas of cherry tomato production, different inoculum sources could be speculated, so a variety of samples were taken: 1) soils from home gardens with vegetables and olive trees for self-consumption, 2) soils from the tools of the tractors used to tillage the soils, 3) plantlets from the nurseries, 4) seeds from 4 different cultivars plus a rootstock, 5) water from the irrigation pools. All analyses were carried out to detect the presence of *Phytophthora*.

**1. Soil and Root Ball Analyses.** A bait plant material technique was used. Trap material consisted of five immature carnation petals. From each sample, three replicates were used. Each replicate consisted of a little tablespoon of sample plated on a 9 cm-diameter petri dish, and merged with a 1 cm-high layer of autoclaved distilled water. Then the carnation petals were laid over the water surface, and the dish tapped. Incubation lasted 7 days at laboratory conditions. Different samples were analyzed by this technique: soil fixed to the root of plants from 34 different home gardens, soil fixed to the wheels and blades of 8 different tractors, root balls of 307 cherry tomato seedlings sampled just when they arrived to the farm in 15 trays from different nurseries.

**2. Seed Analysis.** A total of 863 seeds from five different batches were analysed using the Ulster technique by plating the seeds on potato dextrose agar (Tello et al., 1991).

**3. Pools Analyses.** A similar same bait technique to that explained before for soils was used for waters. In this case, the petals were introduced in sterile jars with little holes wherein the water could go through. Then the jars, fastened by a rope, were introduced in the pools, and remained there for 48 h. Once finished the sampling, the petals were transferred into 20 ml sterile water in sterile petri dishes in the laboratory. During one week the petals were examined for the presence of *Phytophthora*. Ten jars were used per pool. Twenty-nine pools were sampled in two different moments, including pools from the coastal production area. A total of 580 analyses were done.

### Preservation of the Pathogen in the Soil

Forty-two fields were sampled during 2003 and 2004. In the laboratory the samples were dried at laboratory temperature. Then they were introduced separately in plastic bags and sealed with a knot. These soils have been kept in the laboratory until present, out of light and with temperatures between 15 to 28°C. Periodically, samples had been analysed by the bait method explained before.

## RESULTS

### Inoculum Sources

**1. Seedlings from Nurseries.** Only one seedling root ball from 307 samples was positive for the presence of *P. parasitica*. The sampling method consisted in taking the seedling root ball from the trays when arrived in the track from the nursery, due to the absence of symptomatic seedlings. Only one sample was taken from a tray previously laid on the field soil, and this is the unique positive sample. So it seems that seedlings had not carried the pathogen from the nursery.

**2. Seeds.** *Phytophthora* was never isolated from none of the 227, 227, 122, 123 and 124 analysed seeds from 5 different commercial batches.

**3. Home Garden and Tractor Soils.** The plants cultivated in those home gardens were commonly olive trees, sweet peppers, tomatoes and flowers. In Table 1 the presence of *P. parasitica* can be observed in 14.7% of the total gardens sampled. Only one sample from the wheels of one tractor resulted positive for *P. parasitica*. *Phytophthora cryptogea* was also trapped from the soil of one home garden.

**4. Water from Irrigation Pools.** From the total of 576 samples, *Phytophthora* sp. was isolated just from two pools in the new cropping area, and from 3 pools in the coastal traditional-cropping area. The identification of these isolates was not realized because of

the difficulties to purify the isolates from the petals.

### Preservation of the Pathogen in the Soil

These results are presented in two tables (Table 2 and 3), referring to samples taken in two different years. All samples presented *P. parasitica* when they were analysed just after sampling, and 57 months later two samples from a specific area still allow the isolation of the oomycete (Table 2). One of these samples was the unique from a soilless field, and the substrate, perlite, showed the ability to preserve the pathogen. In Table 3 it can be observed how 14 samples preserved the fungi 48 months after the first isolation. This is a long time if it is taken in consideration that the samples were lacking in moisture and host during all this time.

## DISCUSSION

The knowledge about the primary inoculum sources it is essential to develop efficient control strategies for *Phytophthora* (Granke and Hausbeck, 2008). In this work different possibilities have been studied for *P. parasitica*, and different results have been obtained. At first, tomato seeds and seedlings can be discarded as a common way to introduce *P. parasitica* in the studied areas. However, the origin of the contamination can be in the irrigation water as was described by Granke and Hausbeck (2008) for *P. capsici*, Kong et al. (2008) for *P. irrigata* and *P. hydropatica*, Hong et al. (2008) and Theman et al. (2002) for *P. citricola*, *P. citrophthora*, *P. dreschleri*, *P. irrigata*, *P. megasperma*, *P. nicotianae* and *P. tropicalis*. And the other inoculum source seems to be the soil of home gardens, where different plants from different origins are mixed, and the soils are tilled with the same tractors and tools that are used in the cherry tomato fields. About the preservation of *P. parasitica*, Erwin and Ribeiro (1996) present a deep revision about the preservation of different *Phytophthora* species in the soil, enhancing the importance of the fungal structures involved in the persistence of the oomycete. Six years persistence in soil have been reported for *P. parasitica* oospores and chlamydospores in sterilized soils and 4 years in non sterile. In our work, considering that soils were not disinfected, the persistence of the oomycete could be due to both chlamydospores and oospores, because the study of many *P. parasitica* isolates from these areas has revealed the presence of chlamydospores and heterothallic strains (Tello, data not published). With these results, crop rotation does not seem an effective recommendation, but good irrigation criteria and soil management can reduce the dispersion of the disease.

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## Tables

Table 1. Presence of *Phytophtora parasitica* in the soils of home gardens from two new crop areas.

| Crop area | Home gardens sampled | Number of soil samples analysed | Home gardens with *P. parasitica* |
|---|---|---|---|
| Los Bermejales | 18 | 18 | 2 |
| El Negratín | 16 | 50 | 3 |

Table 2. Presence of *Phytophthora parasitica* in soil samples previously cropped with cherry tomato and preserved dried in the laboratory. Year 2003 samples.

| Crop area | Number of samples analyzed | Preservation time (months) | | |
|---|---|---|---|---|
| | | 17 | 29 | 57 |
| El Negratín | 11 | 4 | 2 | 2 |
| Los Bermejales | 2 | 2 | 0 | 0 |
| Albuñol | 1 | 0 | 0 | 0 |

The values represent the number of samples that showed the presence of the fungi.

Table 3. Presence of *Phytophthora parasitica* in soil samples previously cropped with cherry tomato and preserved dried in the laboratory. Year 2004 samples.

| Crop area | Number of samples analyzed | Preservation time (months) | | |
|---|---|---|---|---|
| | | 7 | 19 | 48 |
| El Negratín | 9 | 2 | 1 | 0 |
| Los Bermejales | 68 | 36 | 21 | 14 |
| Almería | 1 | 1 | 1 | 0 |

The values represent the number of samples that showed the presence of the fungi.

## Figures

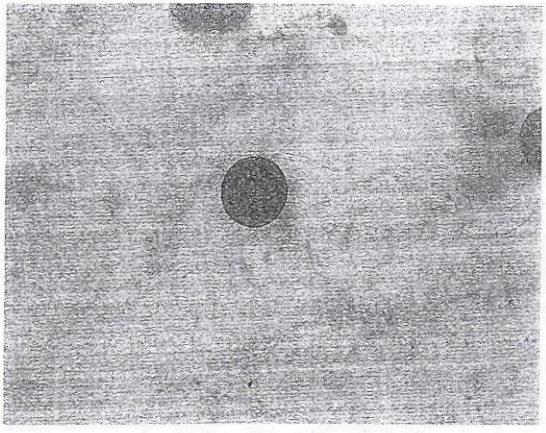

Fig. 1. Chlamydospore of one *Phytophthora parasitica* isolate.